

NON-TECHNICAL ABSTRACT

Severe hemophilia A is a congenital bleeding disorder that results from the lack of a protein in the blood (known as Factor VIII [FVIII]) that is necessary for clotting. Affected individuals are born with a defective gene controlling FVIII production. Because the FVIII gene is located on the X chromosome, virtually all affected individuals are men (X-linked inheritance). Conventional treatment of hemophilia A requires intravenous injection of FVIII to stop bleeding whenever it starts. An alternative treatment approach would be to provide a working copy of the FVIII gene to the individual so that his body could produce FVIII continuously at a level sufficient to prevent bleeding. This would protect him from developing progressive joint disease, pain, and disability—usual complications of hemophilia treated in the conventional manner.

We have produced a retroviral vector that carries a gene for functional human FVIII protein. It is known as the human FVIII retroviral vector. The retroviral vector is a gene delivery vehicle that can enter human cells one time but cannot cause infection or disease, because key components of the virus have been removed or altered. The retroviral vector can carry a FVIII gene into a cell without damaging the cell and make the FVIII gene integrate permanently into the DNA of the cell. Then the gene provides permanent instructions for production of FVIII protein inside the cell and its subsequent export to the bloodstream. This process resembles that by which normal FVIII arrives in the bloodstream of unaffected individuals. The FVIII gene provided in our vector is truncated. This smaller hFVIII gene allows more potent vector preparations to be made, and higher levels of FVIII protein to be produced. Preparations of a similar protein have undergone clinical testing in hundreds of hemophilia patients and these preparations have normal safety and effectiveness.

To evaluate whether a therapeutic amount of FVIII could be produced by treatment with our vector, normal dogs and rabbits received various doses by intravenous injection, and then the amount of human FVIII protein was measured in the blood. Levels of FVIII that lessen disease severity in man were measured in some animals, and persisted for as long as two years. Levels of human FVIII protein varied between animals, but appeared to be higher if more of the vector was injected. In dogs that suffer from severe hemophilia A, treatment appeared to increase the ability of their blood to clot; however, this did not always happen. The variability may well be due to dog antibodies to the foreign human FVIII protein that interfere with the clotting action of FVIII. To test the safety of the human FVIII retroviral vector, we injected rabbits and mice with various doses and monitored them for signs of illness. Treatment with the vector at doses planned for the Phase I clinical trial did not cause changes suggestive of toxicity in body weight, the results of blood tests, or the microscopic or macroscopic appearance of body tissues. At doses about seven times higher than the highest dose planned for human use, two out of ten rabbits died. All mice given doses twelve times higher survived. In animals, very sensitive methods were used to determine which organs contained traces of the vector. Genes from the vector were found primarily in the liver and spleen, (organs which are known to normally produce active FVIII), with very small amounts occasionally detected

in other organs, including the testis. The type of cells containing the vector-derived genes in each of these organs is not known.

The next step in testing the human FVIII retroviral vector will be a Phase I clinical study, primarily designed to evaluate whether administration is safe in humans at doses within the range shown to be safe in animals. Also, blood levels of FVIII will be measured to evaluate whether FVIII is produced at levels potentially protective against bleeding. The goal would be to reach at least 7% of the normal blood level, sustained for at least 12 weeks. This level would be expected to prevent day-to-day bleeding that would otherwise commonly occur. Any bleeding episodes during the study period will be treated as usual with FVIII infusions and reported on home diary forms. Participants in the initial Phase I study are required to have a diagnosis of severe hemophilia A (defined as $< 1\%$ FVIII), to be adults over 25 years of age, to have no inhibitor antibody to FVIII, and to be sterile. If they are HIV positive, they must not be severely immunosuppressed and, if they are positive for hepatitis C virus, they must have no signs of liver failure. The first subjects receiving the vector would receive the lowest dose and then be monitored for harmful side effects for at least 4 weeks before additional subjects are treated. Each participant would receive a single dose, administered intravenously into an arm or hand vein, in three equal parts on each of three successive days. After the highest dose is reached, all subjects will continue to be monitored closely for approximately one year, and then less intensively in a lifelong surveillance registry to evaluate long-term safety. Patients will be monitored for any allergic reactions or other change in physical condition during or after administration, changes in blood tests reflecting liver, kidney, or blood function abnormalities, appearance of an antibody in the blood which would block FVIII function (known as a FVIII inhibitor), and appearance in the blood of replication competent retrovirus (RCR). If any participant developed a positive RCR test or if more than one developed an inhibitor that interfered with treatment for bleeding, then the study would be stopped immediately and no additional subjects would receive the vector.